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HAEMOTROPHIC MYCOPLASMAS IN SOUTH AMERICAN CAMELIDS IN SWITZERLAND

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Summary

The red blood cell parasite 'Candidatus Mycoplasma haemolamae', formerly Eperythrozoon, is known to be widespread in South American camelids in the USA, causing anaemia in affected animals. Up to now, haemotrophic mycoplasmas were not observed in South American camelids in Europe; however, they were known in other species. PCR analysis of blood samples made it possible to find haemoplasmas in a herd of alpacas in Switzerland and to identify them as 'Candidatus M. haemolamae'. Possible ways of transmission are discussed.

Introduction

Haemotrophic mycoplasmas (aka haemoplasma) are small, cell wall-free bacteria parasitizing extracellularly on red blood cells. They were formerly known as *Haemobartonella* and *Eperythrozoon* species and grouped into the order of Rickettsiales; recently, they were reclassified within the genus *Mycoplasma* based on phylogenetic analyses of the 16S rRNA gene (MESSICK et al., 2002). The *Eperythrozoon* species previously identified in South American camelids (SAC) was renamed '*Candidatus* M. haemolamae'. The latter agent is most closely related to *M. wenyonii* and *M. suis* affecting cattle and pigs, respectively.

Clinically, haemoplasmas cause anaemia in acutely infected individuals. Disease outbreak can also occur in chronically infected animals in conditions of stress and/or immunosuppression. A chronic carrier status following acute '*Candidatus* M. haemolamae' infection has been reported in SAC. Syndromes like chronic weight loss and weakness in alpacas and llamas have been suspected to be caused by these chronic infections, but healthy carrier animals have also been observed (TORNQUIST, 2002). However, these animals can represent a source of infection for their herd mates. Arthropod vectors have been suspected as natural means of transmission of haemoplasmas between SAC, but this has neither been proven yet nor was a vector identified. As this point has not yet been elucidated in SAC, transmission via blood-contaminated instruments, shearing machines or Gigli's wire is likely too. Treatment of clinically ill animals includes parenteral or peroral tetracycline application. However, standard tetracycline regimen does not seem to eliminate the haemoplasmal infections in all infected animals (TORNQUIST, 2002).

Infections of SAC with '*Candidatus* M. haemolamae' are well known and widespread in the USA, but to our knowledge have not been detected in Europe yet. In applying PCR-based diagnostic methods we recently detected '*Candidatus* M. haemolamae' infections in a herd in Switzerland.

Animals, material and methods

All the cases presented in this study originate from a herd composed of 140 SACs (number at first investigation). The animals are kept in 6 groups on different pastures. Most of these animals were imported from Peru in 2004. The herd is located in the southern part of Switzerland on an altitude of 850 to 1000 meters above sea level. The animals live on pasture with open shelters during the whole year. Feed consists of grass, hay and a spot of grain and mineral salts are freely available. Intestinal parasites are controlled by regular coprological monitoring and adequate treatment. Normally, there is no exchange of individuals between the groups. For breeding reasons, group 1 has always been strictly separated from the other groups from the date of importation on. Distance between the pastures of the single groups ranges between 50 and 750 meters (air-line distance). At the time of investigation, group 1 was separated by 500 meters to the next other group.

Two necropsies including histological examinations of organs were performed at the Institute for Veterinary Pathology, Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Berne, Switzerland. Faecal samples for parasitology were submitted to the Institute for Veterinary Parasitology, Vetsuisse Faculty, at the University of Berne, Switzerland. EDTA-anticoagulated blood samples from venipuncture were sent to the Laboratory of the Department for Clinical Veterinary Medicine Vetsuisse Faculty of Berne, Switzerland for haematological analyses. Either whole blood samples from the heart (in case of necropsies) or EDTA-anticoagulated blood samples from venipuncture were sent to the Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland. Samples were analysed for '*Candidatus* M. haemolamae' infections by conventional PCR assays developed for the detection of *M. wenyonii* in cattle (MELI ET AL. manuscript in preparation). The primers of the latter assay showed no mismatch when aligned to a published '*Candidatus* M. haemolamae' 16S rRNA gene sequence (AF306346). To additionally confirm '*Candidatus* M. haemolamae' infection, the PCR amplification products were sequenced and compared to the published sequence. Thereby, 98 % identity was found.

Cases, results and discussion

In June 2006, a female alpaca of group 1 was referred to the Institute for Veterinary Pathology, Centre for Fish and Wildlife Health in Berne. She had died unexpectedly 5 days after giving birth to a foal; the cria died two days after birth. On gross necropsy of the dam, emaciation and paleness of the body were evident. Exudation was found in all body cavities, the kidneys were swollen and the liver had scattered, mineralised white spots. In all compartments of the stomach, multifocal to extensive reddish areas were seen. The faeces in the rectum were smooth. No other changes were observed in the other organs. Histologically, the white spots in the liver consisted of mineralised granulomas containing eggs of *Dicrocoelium dendriticum*. In compartment 1, a focal extensive ulceration contaminated with bacteria colonies and flagellates was observed. Additional histological finding consisted of presence of protein casts in the renal tubules and a slight depletion of lymph nodes. Parasitological investigation revealed *Eimeria spp.*, *Dicrocoelium dendriticum* and Nematode eggs in the faeces. A sample of heart blood was analysed for '*Candidatus* M. haemolamae' and found to be PCR-positive.

One month later, the body of another female from group 1 was referred to the Centre for Fish and Wildlife Health. She had given birth to a cria one month before, which died also for unknown reasons. Clinically, the dam showed weight loss, extensive skin alterations typical of sarcoptic mange and pale mucous membranes. Gross necropsy revealed cachexia, anaemic mucous membranes and thickened, crusty skin covering most of the body. The liver had mineralised, whitish spots and

Dicrocoelium dendriticum were observed on the cut surface gushing out the biliary ducts. The lymph nodes were swollen and the uterus was filled with a mucopurulent secretion. Histologically, corresponding alterations were a purulent inflammation of the endometrium, marked neutrophilic infiltration of the lymph nodes, hyper- and parakeratosis containing numerous arthropod mites and associated with deep eosinophilic inflammation of the dermis and severe pulmonary edema. Mites were isolated from the skin lesions and identified as *Sarcoptes* spp. Coprological analysis revealed the presence of *Dicrocoelium dendriticum*, *Eimeria spp.*, *Nematodirus* and *Trichuris*. In this animal, an infection with *'Candidatus* M. haemolamae' was also confirmed by PCR analysis using heart blood.
Based on these results, the decision was taken to investigate the herd's epidemiological status for *'Candidatus* M. haemolamae' infection by random sampling in different groups. In July and August 2006, a total of 23 blood samples were collected from 21 alpacas and two llamas. Signalement, haematological data and PCR results of all examined animals are listed in Table 1. Remarkably, 10 out of 23 animals (43.5 %) tested PCR-positive for *'Candidatus* M. haemolamae'; no llama was found to be infected. All except one alpaca in group 1 were infected. However, none of these animals showed signs of disease nor was anaemia identified by haematological analyses (Table 1). A treatment trial was initiated in order to eliminate the infection from the animals which were known to be infected. Nine out of 10 positive animals were treated with oxytetracycline (Engemycin®, 20 mg/kg s.c. SID for 10 days). Two weeks after the treatment had been suspended, the alpacas were retested by PCR, and four still tested positive. Therefore, the latter four animals were again treated for 10 days with the above mentioned regimen and retested two weeks after final treatment. Still three alpacas tested PCR-positive. At this point, no further treatment was performed. However, the animals showed neither clinical illness nor anaemia. The owner was instructed to closely observe the infected animals especially in conditions of unusual stress and around parturition and to resume treatment in case of exacerbation.

Tab. 1: Signalement, PCR results, haematocrit values and group affiliation of SAC examined for 'Candidatus M. haemolamae' in July and August 2006.

Animal No.	Group	Species	Sex	PCR Result	Hematocrit (%)
6	1	Alpaca	f	pos	30.7
7	1	Alpaca	f	pos	27.2
12	4	Alpaca	f	pos	28.7
19	1	Alpaca	m	pos	31.2
35	4	Alpaca	f	neg	29.8
50	2	Alpaca	f	(pos)	36.1
54	2	Alpaca	f	neg	44.3
56	4	Alpaca	f	neg	27.8
61	4	Alpaca	f	neg	35.1
66	1	Alpaca	f	pos	29.3
Foal of 66	1	Alpaca	m	neg	31
73	1	Alpaca	f	pos	33
96	1	Alpaca	f	neg	31.4
194	1	Alpaca	f	pos	30.2
Foal of 194	1	Alpaca	m	neg	31.8
196	1	Alpaca	f	pos	29.7
204	2	Alpaca	f	neg	31.2
216	1	Alpaca	f	neg	19.7
230	1	Alpaca	f	neg	29
351	2	Alpaca	f	pos	37.8
431	3	Lama	f	neg	41.9
432	3	Lama	f	neg	43
722	1	Alpaca	m	neg	41.2

Infections with *'Candidatus* M. haemolamae' are often reported be associated with depression, lethargy and chronic weight loss. However, little is known about the real pathogenic potential of the organism in SAC (TORNQUIST, 2002). The two animals in our report that died after parturition suffered from other infections and parasites. Therefore, it is difficult to determine the exact significance of '*Candidatus* M. haemolamae' infection in these cases. Unfortunately, no data were available concerning the clinical and haematological status of these two animals prior to death. However, the alpaca did show signs of anaemia attributable to *'Candidatus* M. haemolamae' infection. Haemoplasmas are reported as exacerbating infections especially under stress and/or in juvenile immunosuppressed animals (MESSICK et al., 2004). Parturition in the above mentioned females together with the observed parasite infestation could thus have led to a fatal outcome of haemoplasma infection. A different pathogenic potential of *'Candidatus* M. haemolamae' infection in debilitated compared to healthy animals was also supported by the finding that 10 out of 23 animals of the examined SAC tested PCR-positive without exhibiting any clinical signs attributable to infection. Although haemoplasmas will well cause chronic disease in different host species (MESSICK et al., 2004), this is not sure for SACs. Chronic carrier state can last for months or even years. As a PCR-positive result does not imply an acute infection but could also arise from a chronic carrier status, we can only speculate about the exact date of haemoplasma infection in these animals. As group 1 showed the highest infection rate, it can be assumed as the origin of the infection; fortunately, these animals did not have any direct contact to SAC of other groups. It is well possible that the animals or at least part of them carried the organism since their importation date in 2004. However, the way of haemoplasma transmission remains to be elucidated. Arthropod vectors have not been evident on these pastures. According to the information of the owner, all instruments for shearing, hoof trimming etc. are disinfected before used for animals of different groups. One of the necropsied females showed severe *Sarcoptes* infestation; theoretically, these mites could have served as vector for *'Candidatus* M. haemolamae' and could be transmitted indirectly by care-taking persons e.g. A potential vertical transmission of haemoplasmas could have led to infection of the foals in group 1. An in-utero infection with *'Candidatus* M. haemolamae' was recently reported (ALMY et al., 2006). In 3 out of 11 animals that repeatedly tested PCR-positive, two subsequent treatments could not resolve infection. Although tetracyclines are effective against haemoplasma induced diseases and reduce the bacterial loads in infected animals, no antibiotic treatment has so far been found to consistently eliminate infections. Some authors however recommend continuing antibiotic treatment for up to 50 days to increase the chance to eliminate the organisms (MCLAUGHLIN et al., 1990).

This is the first report on *'Candidatus* M. haemolamae' infections in alpacas in Europe. The agent has to be considered as differential diagnosis in case of anaemia or other chronic debilitating diseases in SAC in our countries. With regard to the pathogenic potential of this agent, especially in immunosuppressed or debilitated animals, the prevalence of infection in SAC in Europe and the impact on the health status on herd levels should be further investigated.

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